CHEMICAL CHANGES IN TUBERCULOUS TISSUES

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In this study of the chemical composition of tuberculous tissues, the lack of uniformity in the methods used by different investigators made it imperative, for the sake of comparative results, to make analyses of normal as well as tuberculous tissues. Since large specimens were required, bovine tissues were used chiefly as large amounts of tuberculous material were available in an extensive packing center. Lymph gland and liver tubercles were used exclusively for the tuberculous specimens and normal lymph glands and livers furnished the materials for comparison. Such a comparative study reveals some of the more marked changes in the tissues, resulting from the reaction to the tubercle bacillus.

Numerous more or less complete analyses of the livers of men and of animals have been reported. The water and the fats contained in the liver received the greater share of attention from many of the earlier workers. v. Bibra[1] as early as 1849 gave 76.19% as the average water content of 6 approximately normal human livers. The amount of fat in these livers is given as 2.86% of the moist weight, or about 12% of the dry weight. Similar determinations on bovine livers gave the water content of 2 specimens as 70.86 and 71.92%, with fat percentages of 2.64 and 3.28, or calculated on the dry weight, the average percentage of fat is 10.35. In a more recent study of normal bovine liver, Profitlich[2] reported the average percentage of water in 7 different livers as 71.66. The fat content of these livers seems to have varied widely, ranging from 10.87-21.78% of the dry weight, with an average of 16.75%. The ash determined for one of these livers constituted 3.83% of the dry weight.

In connection with his study of the human liver in acute yellow atrophy and in chloroform poisoning, Wells[3] made careful analyses also of the normal human liver. He gives 77.6% as the amount of water in the normal human liver and 5% of fat. Lecithin and cholesterol determinations were made on the lipin fractions obtained from the livers of 2 persons who died suddenly. The lecithin averaged 31.7% of the ether-soluble substance, or 6.3% of the dry weight; cholesterol formed 6.7% of the ether-soluble material, or about 1.3% of the dry weight. The total amount of lecithin in the liver in acute yellow atrophy was very greatly reduced, not only as to the actual amount present, but also in relation to the other constituents of the liver. It formed

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[1] Quoted by Von Gorup-Besanez, Lehrbuch d. physiol. Chem., 1878, p. 711.
[2] Arch. f. d. ges. Physiol., 1907, 119, p. 465.
[3] Jour. Exper. Med., 1907, 9, p. 627; Jour. Biol. Chem., 1908-9, 5, p. 129.

17.6% of the ether-soluble substances, or 2.9% of the dry weight. A decrease in lecithin was also noted in delayed chloroform poisoning, although fatty changes were moderate in degree, but the decrease was by no means so marked as in acute yellow atrophy. The amount of cholesterol in the liver in acute yellow atrophy was not so greatly reduced, in fact, the total amount for the entire liver was about the same as that found in the controls, although it constituted a larger percentage of the ether-soluble substances. This is accounted for by the reduction in the amounts of neutral fats and lecithin.

In chloroform necrosis, the increase in the fat content of the liver was found to be due entirely to simple fats. As a continuation of the analyses of human liver, Wells studied also the alcohol-ether-insoluble fraction. The residue left after extraction with alcohol and ether was extracted in a shaking machine repeatedly with fresh quantities of water, and then with water at 50-60 C. The residue of liver tissue was then extracted with boiling water. The amount of gelatin in the hot water extract was determined and the remaining solids, obtained by evaporating the filtrate, were added to the united cold and warm water extracts. After the concentration of these extracts, the proteose-peptone mixture was precipitated by means of alcohol. Amino-acids and purins were determined in the nonprotein portions of the watery extracts. The residue of insoluble liver substance left after all the extractions had been completed, was dried to constant weight and analyzed. The composition of the coagulated and insoluble proteins of the liver after thorough extraction with alcohol, ether, cold and hot water, was found to be quite the same in chloroform necrosis and in acute yellow atrophy as in normal livers.

Determinations of insoluble sulphur, phosphorus and iron were made on the extracted residues. The sulphur was practically constant in all 4 specimens, in spite of the great structural changes in the 2 diseased livers. The insoluble phosphorus, however, in the acute atrophy and chloroform necrosis livers was, in each instance, increased to about 4 times the amount present in the normal livers.

The increase in the phosphorus in acute yellow atrophy is explained as the result of the great proliferative activity exhibited by the cells of the stroma and bile ducts in areas where regeneration is taking place, giving rise to large numbers of new cells rich in nucleic acid. The phosphorus found in the normal livers constituted 0.24% of the extracted residue, in acute yellow atrophy and in chloroform necrosis the phosphorus was 0.90% of this residue. The amount of sulphur ranged from 0.75-0.82% of the extracted material in the normal and pathologic livers. Heffter[4] studying the liver in relation to phosphorus poisoning, made lecithin determinations on the livers of normal rabbits and found that it constituted about 2.2% of the dry weight. In another series of rabbits which had been poisoned with phosphorus, the average lecithin content dropped to 1.12%, or about ½ that of normal livers. Baskoff[5] made lecithin and jecorin determinations on normal dog livers and on the livers of dogs poisoned with alcohol. In the normal animals, the phosphatids constituted 8.4% of the dry weight, while in animals which had been poisoned with alcohol for 9 months, the phosphatids had decreased to 3.9%. The jecorin in the normal livers was 14.4% of the total phosphatids, while in the animals poisoned with alcohol it had increased to 39%.

[4] Arch. f. exp. Path. u. Pharm., 1891, 38, p. 97.
[5] Ztschr. f. physiol. Chem., 1909, 62, p. 162.
[6] Quoted by von Gorup-Besanez, Lehrbuch d. physiol. Chem., 1878, p. 732.

Normal lymph glands, either of human beings or of the lower animals, have evidently never been made the subject of as careful studies as those recounted for the liver. In the inguinal glands of an old woman, Oidtmann[6] reported the finding of 71.43% of water, 28.45% of organic material and 0.12% of inorganic substances. In the mesenteric lymph glands of oxen, Bang[7] reported 80.41% of water, 19.59% of solids, 13.79% of total proteins, 0.69% of histone nucleinate, 1.06% of nucleoprotein, 4.76% of substances soluble in alcohol and 1.05% of mineral constituents. The structurally related thymus gland has been subjected to accurate chemical studies but here the chief interest has usually been centered in the nuclein substances. Lilienfeld[8] analyzed the cells of the calf thymus and reported a total phosphorus content of 3.01% of the dry weight; the total nitrogen was 15.03%, lecithin 7.51%, cholesterol 4.40%, fat 4.02%, and the silver salts of the nuclein bases 15.17% of the dry weight. The dry weight of these cells amounted to 11.49% of the fresh weight. An analysis of the human spleen by Burow[9] and Magnus-Levy[10] showed the water content of that organ to be 78.4%, the solids 21.53%, the fat 2.77% and nitrogen 2.79%, all calculations being made on the fresh weight of the organ. A complete analysis of the dog spleen was made by Corper.[11] The averages of the analyses of 3 normal spleens gave a moisture content of about 75-77% and a content of ether-soluble materials between 11.6 and 15.5% of the dry weight. The ether-soluble fraction was made up of about 1.5% of cholesterol, and between 6 and 7% of lecithin, leaving 2-6.5% for neutral fats. The total soluble nitrogen ranged between 0.45 and 0.97% of the dry weight and was about equally divided between that precipitable with tannic acid and that which could not be so precipitated. The water-soluble phosphorus content was about 0.27-0.52%. With the amounts of tissue used, no purins could be identified in the water-soluble fraction. The insoluble part of the tissue contained about 0.26-0.98% of dry weight as iron, 0.53-0.60% as sulphur and about 0.39% as phosphorus, with a purin nitrogen content of 0.24-0.35% of the dry weight. The total nitrogen content of the insoluble part was about 11-13% of the dry weight. Of the purin enzymes, evidence was obtained of the presence of xanthin oxidase, adenase and guanase, while uricase was lacking.

The nature of the changes occurring in tissues during the caseation produced by infections with the tubercle bacillus has been the subject of much speculation and has led to numerous investigations limited chiefly to the fatty constituents. On the basis of the gross appearance and, likewise, from the ordinary microscopic preparations, it seemed evident that caseous material was rich in fats; that it consisted chiefly of globules of fat and granules of coagulated protein. It was thought, perhaps, that the tubercle bacillus which seems to be able to synthesize fat when grown on glycerin agar, might produce somewhat similar changes in the necrotic area and its vicinity, giving rise in this way to the fatty changes of caseation.

Schmaus and Albrecht,[12] having studied caseation necrosis microscopically, state that the process consists of the death of the cellular elements and the origin of a firm intercellular substance arising as a transudate, together with the precipitation of a fibrinoid material, probably not identical with fibrin but giving the typical reaction of fibrin. The formation of the caseous detritus

[7] Hofmeister's Beiträge, 1903, 4, p. 115.
[8] Ztschr. f. physiol. Chem., 1894, 18, p. 473.
[9] Biochem. Ztschr., 1910, 25, p. 165.
[10] Biochem. Ztschr., 1910, 24, p. 363.
[11] Jour. Biol. Chem., 1912, 11, p. 27.
[12] Virchows Arch., 1896, 144, p. 72, Suppl.

follows by a progressive breaking up of the fibrinoid material. To these substances are attributed the chief importance in the formation of the firm dry condition of the caseous mass. Along with the production of the intercellular substance goes the disappearance of the chromatin of the cell nuclei. Using fat stains, Rosenthal[13] found no evidence of fat in miliary tubercles unless there was some caseation present. The fat seems to make its appearance with the occurrence of the caseation. Within an extensive caseous area no fat was found, or at most only traces, while just at the boundary of the caseous area, fat-bearing cells were seen. Some of the giant cells in this region are said to resemble fat cells, while other giant cells were apparently fat-free. Streaks containing small fat droplets were sometimes seen in sections cut through fresh caseous areas. These are explained as representing the boundaries of small caseous areas which have fused to form the larger area, in which case the fat had not disappeared as it seems to do with the gradual extension of the caseation in the tuberculous tissue. Likewise, Vallillo,[14] in studying avian tuberculosis, observed that non-necrotic tubercles composed of epithelioid and giant cells contain either no stainable fat or only sparsely grouped fat droplets in the center. In the tubercles which had necrotic centers, the fat droplets were abundant and were located chiefly in the cytoplasm of the giant cells accumulated there. The fat droplets, however, were not numerous in the caseous part of the tubercles.

Similar observations were made by Hagemeister[15] He noted that in large conglomerate tubercles, fat droplets were not infrequently observed within the tuberculous area close to the margin of the older individual tubercles. In other cases, the boundary zone apparently contained a large amount of fat. Sometimes, in the caseous tubercles, two or even three of these fat-containing marginal zones were noted at regular intervals, an occurrence explained as being due to the advance of the necrosis in successive stages. Even in the necrotic areas the outlines of the giant cells could sometimes be made out by the arrangement of the fat droplets. Herxheimer,[16] studying lung and lymph gland tubercles for the occurrence of fat, confirmed the findings of Rosenthal and Hagemeister. Chaussé,[17] using sudan III and osmic acid, demonstrated fat droplets in the giant cells and in practically all other cells in the tuberculous area and also in the substance derived from the destruction of all these cells. In a study of necrobiotic fatty changes, Joest[18] used fat stains on tuberculous tissues with results quite similar to those previously reported by Rosenthal. He was never able to demonstrate fat in the intercellular substances. Emphasis is placed on the observation that although fat is demonstrable in the caseous part of the tubercle, it is less prominent there than in the boundary zone of the living tissue, and the living tubercle tissue apart from the boundary zone is free from stainable fat. It is explained, however, that this impression of a lesser fat content in the caseous portion is, doubtlessly, due in part to the fact that the fat in the living cells of the boundary zone occurs in sharply circumscribed globules, while, in the caseous area, the globules are broken up and the fat more diffusely scattered.

[13] Verhandl. d. deutsch. path. Gesellsch., 1899, 2, p. 440.
[14] La Tuberculosi, IV, 257, 1911-2.
[15] Virchows Arch., 1903, 172, p. 72.
[16] Ergeb. d. allg. Path. u. path. Anat., 1902, 8, p. 669.
[17] Compt. rend. Soc. de biol., 1909, 64, p. 377.
[18] Virchows Arch., 1911, 203, p. 451.
[19] Jour. Med. Research, 1905-6, 14, p. 491.

In the study of pathologic calcification, Wells[19] used both staining and chemical methods for the recognition of fats in tuberculous tissues. Specimens of human and bovine tuberculous lymph glands, stained with sudan III, revealed marked infiltration with fine and coarse fat granules all through the areas that are acellular, the largest and most abundant granules being usually at the periphery. When counterstained with hematoxylin, the calcium deposits were found to lie in such tissues as were stained for fat, but there was no particular difference to be noted in the amount or character of the fat in the vicinity of the calcium deposits and elsewhere. Not infrequently a calcium deposit was noted at the periphery of the gland or tubercle, while, in the center, there was no calcium but many fat granules, although not more than in the tissues surrounding the calcium deposits. For the chemical studies, Wells used tuberculous mediastinal lymph glands of cattle. He calls attention to the fact that bovine tuberculous lesions differ from human lesions in that calcification occurs during the progress of the disease and is extensive in the form of innumerable sandlike granules, scattered all through the tuberculous tissue even while the disease is in the most active stages. Calcification is usually an evidence of latency or healing in human tuberculous areas, and the deposits are found in much larger masses, each of which usually corresponds to an entire tubercle. Two sorts of bovine material were collected and examined separately in the course of his investigations. One consisted of the fluid puslike content of the large softened glands. This material escapes when the glands are opened and contains but few granules of calcium large enough to be felt by the finger. The other specimen was obtained by scraping the surface of unsoftened tubercles and the walls of the tubercle cavities. It consisted largely of the calcified material and the adherent tissue, mixed with more or less of the tissue elements but giving a fair conception of the substances immediately about the calcified masses. After drying this tuberculous material, it was thoroughly extracted with ether, alcohol and amyl alcohol. The inorganic salts of calcium were quite insoluble in these solvents. The residues left from these extractions were then extracted with large volumes of water and calcium, magnesium, phosphorus and carbon dioxid determinations made on the water-insoluble residues. The total lipin content of the scrapings from the walls of the calcified bovine lymph glands was found to be very appreciably higher than that of the caseous liquid content of these lymph gland tubercles. Since these values, in either case, are based on the dry weight, this difference is dependent in no direct way on the water content. Likewise, the low lipin content of this dried caseous material cannot be attributed to the presence of the heavy calcium salts, for both the calcium and the phosphorus are present in only about ⅓ of the amount found in the scrapings from the tubercle walls. The lipin content of calcified human tuberculous lymph glands is low, but in this case the calcium and phosphorus values are extremely high, showing that the dry weight here is made up in large part of calcium salts. The MgO was found to hold a constant relation to the CaO and the amount present was always small; likewise, a rather definite ratio existed between the amounts of carbon dioxid and of CaO. The water-soluble fraction of the caseous liquid content of the tubercles constituted a smaller percentage of the dry weight than it did in the scrapings from the walls of these tubercles. The water-soluble materials obtained from the calcified human tuberculous glands was still much smaller in amount. In the latter case this might be due to the presence of great amounts of relatively insoluble inorganic salts.

[20] Zur Chemie der Verfettung, Dissertation, Basel, 1902. Quoted by E. Schmoll, Deutsch. Arch. f. klin. Med., 1904, 81, p. 163.

Perhaps the most complete of the analyses which have been made of the lipin fraction in tuberculous caseous material are those which were made by Bossart.[20] The materials used were of human origin and only 1 consisted of completely caseous material, the remaining 4 specimens ranged from 1/6-1/3 caseous substances obtained from lymph glands. The reported fat content in percentage of dry weight varied from 13.77 in a specimen which was estimated as 1/6 caseous to 23.79 in a specimen 1/4 caseous. The total fat content of the pure caseous material is given as 20.75% of the dry weight. The figures reported for the lecithin content are apparently of no great value since no lecithin at all was obtained in 3 out of the 5 specimens. In 3 specimens varying from 1/6-1/3 caseous, cholesterol ranged from 25.8-33.5% of the total fats, while the latter are reported as varying from 13.77-15.73% of the dry weight. In the completely caseous specimen, the cholesterol value is given as 2.77% of the alcohol extract, apparently a much smaller amount of cholesterol than that found in the partially caseous specimens. The negative findings for lecithin reported by Bossart have not been substantiated by other workers with caseous material. Schmoll[21] was able to demonstrate considerable amounts of glycerol-phosphoric acid in the alcoholic extracts of all specimens of pure caseous material which he examined. He used 3 specimens of completely caseous material from bovine lymph glands, and 1 specimen of human material which was about 1/4 caseous. The caseous residues left after alcohol-ether extraction were extracted with cold water with the addition of toluene. This was found to be a very troublesome procedure as it was necessary to change the water twice daily for 5 or 6 weeks in order to make the extractions complete. No protein materials seemed to go into solution, as all the protein reactions were either completely negative or scarcely evident. Elementary analyses were made on the residue insoluble in alcohol, ether and water. The calculations made on the basis of the ash-free material gave the following averages for the 3 specimens of completely caseous material: carbon, 53.92%; hydrogen, 7.38%; nitrogen, 16.44%, and sulphur, 0.65%. The ash content varied from 9.2-23.3%. Phosphorus was determined only on the specimen of caseous material containing 23.3% ash and in this case it constituted 1.04% of the ash-free substance. The ash value for the human tuberculous specimen, 1/4 caseous, was 4.63%, with a phosphorus content of only 0.25%. This exceptionally low percentage of phosphorus is stated as being surprising, since it was obtained from tuberculous but not completely caseated lymph glands. As this tissue is normally rich in phosphorus, this finding seemed to indicate that the process of coagulation necrosis, as appears evident also microscopically, is accompanied by a destruction of the cell nuclei and a washing away of the products formed. Whatever the sulphur content means, it was thought to be distinctly lower here than in most proteins. However, the value given is slightly higher than that given by Corper[11] for the sulphur content of the dog spleen. In order to find out more concerning the character of the protein in caseous material, Schmoll studied its conduct toward pepsin-HCl digestion and hydrolytic cleavage with HCl. The fluid obtained by about 2 months digestion with the pepsin-HCl mixture showed that this protein substance differs in no essential way from other proteins so far as its conduct toward digestion is concerned. From the results obtained, Schmoll thought that he could exclude with certainty the existence of any nuclein material whatever, since

[21] Deutsch. Arch. f. klin. Med., 1904, 81, p. 163.

no precipitate was obtained with ammoniacal silver solution and he remarks that the undissolved portion was certainly not nuclear material. Following hydrolysis with HCl, a partition of the nitrogen gave the following values:

Humin and ammonia nitrogen	5.01% of total N
Basic nitrogen	43.9 %
Amino-acid nitrogen	51.1 %

The low percentage of humin and ammonia nitrogen as well as the richness in basic nitrogen is noted as being remarkable. An attempt to determine guanin and adenin was unsuccessful. Schmoll studied also the autolysis of tuberculous caseous material and found that the autolytic processes go on extremely slowly in such tissues. He remarks that this may explain the fact that caseous material is so rarely absorbed. In connection with F. Müller, Schmoll analyzed the lipin fraction of a specimen of caseous material from human lymph glands. Cholesterol was found present but the amount is not stated. The phosphorus content of the ether-soluble material was 1.57%, or when calculated as lecithin, 38.31%. This corresponds to 3.83% of the dried caseous material.

A study of the lipoids and their content in phosphorus in different organs and tissues of guinea-pigs, during chronic tuberculosis, was made by Griniew.[22] He finds that in this disease the composition of the cells of nearly all the organs and tissues changes so far as the lipoid content is concerned. The change is qualitative as well as quantitative and is shown by the diminution in the amount of phosphorus in the lipoids and by the replacement of some lipoids by others. The total quantity of all the lipoids decreases as well. The quantity of cholesterol is increased in the liver, kidney, brain and heart, and decreased in muscles, lung, spleen and bone marrow. There is less lecithin than normal in all the organs, that is, it constitutes a smaller percentage of the total lipins. In nearly all of the organs, the percentage of kephalin is increased. The enzymes of the tuberculous tissues were also studied and the lipolytic power of the tuberculous organs was apparently subnormal. The lungs, liver and kidneys are reported as markedly subnormal in catalase, while the catalase content of the heart is increased.

A comparison of normal human livers with those of 5 persons dying of tuberculosis was made by Robin.[23] The water content was approximately 7.7% higher in the tuberculous livers than in the normal ones and this change seemed to be more marked in the acute than in the chronic forms of tuberculosis. There was only a slight change in the fat content and a minimal lessening of organic sulphur.

Corper[24] used intra vitam staining methods in a study of the fat in the tubercles of guinea-pigs. It had been shown by the work of Riddle[25] and others that fat dyes such as sudan III and scarlet R, entering the body dissolved in fat, remain either entirely or chiefly with this same food fat, being deposited with it if the food fat was deposited, but not leaving the food fat to enter either stored fat or the intracellular fats or lipoids of active tissues. Corper observed that the fats of tubercles never contained any demonstrable amount of the fat dyes administered, no matter whether the tubercles formed before or after the animal was saturated with the dye. He states, therefore,

[22] Arch. des sciences biol., 1912, 17, p. 177 and p. 363.
[23] Ztschr. f. d. ges. Physiol. d. Stoffwechs., 1911, 6, p. 576.
[24] Jour. Infect. Dis., 1912, 11, p. 373.
[25] J. Exper. Zool., 1910, 8, p. 163.

that it seems probable that the fats microscopically visible or chemically demonstrable in tubercles, are derived chiefly or solely from the existing fats and lipoids of the disintegrated cells and are not deposited from the fats in the blood. This view is entirely in harmony with the histologic evidence.

EXPERIMENTAL

Through the courtesy of the Western Packing Company and the Peerless Packing Company of Chicago, large amounts of bovine lymph gland and liver tubercles were obtained, and also fresh normal lymph gland and liver. Both the normal and the tuberculous lymph glands came from the peribronchial and mesenteric regions. The normal peribronchial lymph glands were of the usual size for cattle, the largest being about 6 cm. in greatest dimension. The mesenteric lymphoid tissue occurred in two forms: (1) lymph glands of the usual rounded or oval form with a definite hilus, and (2) long strips of lymphoid tissue, sometimes 20-30 cm. long, about 1 cm. wide and of a corresponding thickness.

From all of these lymph glands, the surrounding fatty tissues were removed with great care until no definite masses of fat were left anywhere on the surfaces of these glands. The fibrous capsules, however, were not removed and, undoubtedly, an appreciable amount of fat was left unremoved. This is noteworthy here, since the method of preparing the specimens from the tuberculous glands was such that this extraneous fat would not enter to contaminate the tuberculous materials, since, in no case, were the tuberculous glands used entirely, but only the walls of the tubercles and the caseous materials from these tubercles. The tubercles in the lymph glands averaged from 3-5 cm. in diameter and their caseous centers were sometimes 2-3 cm. in diameter. The surrounding normal tissues were removed as completely as possible from the tubercles, after which the tubercles were opened and their caseous contents expressed. The walls of the peribronchial lymph glands tubercles were kept separate from the walls of the mesenteric lymph gland tubercles, while the caseous material from all of these lymph gland tubercles was made into one composite specimen, since the amount was not sufficient to divide.

Likewise, the tubercles occurring in bovine livers were separated carefully from the surrounding liver tissue and the caseous material removed from the surrounding fibrous capsule. The largest of these tubercles varied from 1-5 cm. in diameter, while some of the livers were studded elsewhere with many smaller tubercles.

Only the larger tubercles which could be more readily separated from the normal tissues were used for these analyses. Two specimens of caseous material were obtained from tubercles having a diameter of 1-2 cm., and one specimen came from tubercles 2-5 cm. in diameter. Two specimens consisting of liver tubercle walls were preserved for analysis.

The normal and tuberculous tissues were all obtained fresh from the packing house, and all were treated throughout in as nearly as possible the same manner. The normal tissues and the walls of the tubercles were ground fine in a meat chopper, after which samples were removed for the determination of the water content and the dry weight. The caseous material consisted of a semifluid mass in which there was no macroscopic evidence of calcification other than the occurrence of numerous sandlike particles which tended readily to settle toward the bottom of the container leaving the superficial layers more distinctly fluid. The caseous material from the largest liver tubercles was more watery in its appearance than that from the smaller tubercles. Samples of the caseous material were, likewise, removed for the determination of the dry weight. The specimens of the normal and the tuberculous tissues, selected in such a way as to contain approximately 100 gm., were then weighed and about 5 volumes of 95% alcohol, containing a minimum of nonvolatile substances, were added. The specimens were then placed on a steam bath where they were allowed to remain for an hour at a temperature closely approximating the boiling point of the alcohol. Some of the specimens were used for immediate analysis, while the others preserved as indicated were kept for subsequent use.

General Plan of Analysis.—(*a*) Water content and dry weight of tissues. Amounts of the freshly ground tissue, varying from 0.5-1.5 gm., were weighed in appropriate weighing bottles and, after some preliminary drying at a lower temperature, were placed in an electric oven which was maintained at a temperature of 90-100 F. A practically constant weight was obtained by heating 48-72 hours. Some of the tissues in the weighing bottles were first treated with several volumes of 95% alcohol, which partially dissolved out the fats and rendered the tissue more porous so that a constant weight could be obtained with a briefer period of heating.

(*b*) Extraction of lipins.

When one of the preserved specimens was selected for analysis, it was first heated on the steam bath and the supernatant alcohol containing a part of the lipins was filtered through the extraction cup which was to be subsequently used in the Greene extraction apparatus. The specimen was extracted 3 times in this way with redistilled 95% alcohol. The residue was then placed on a watch glass and allowed to dry, first at room temperature and then in

the electric oven at a temperature of 90-100 F. The dried tissue was then ground in a mortar and transferred to the extraction cup which had been used previously as a filter. It was next extracted for 24 hours with hot absolute alcohol and then for 24 hours with redistilled ether. The residue was then removed from the extraction cup and the ether allowed to evaporate. The residue so obtained was once more ground in a mortar and the powdered tissue again extracted for 24 hours with absolute alcohol. All of these extraction fluids were united in a measuring flask of 500 or 1,000 c c capacity. In the early part of this work all of this lipin solution was evaporated to dryness at a temperature of 50-60 C. under reduced pressure and the total lipin fraction determined by weighing the residue so obtained. When this procedure was followed, after a nearly constant weight had been obtained in a vacuum desiccator, this lipin fraction was emulsified in water and transferred to a measuring flask of 500 or 1,000 c c, depending on the amount of fatty material obtained. Most of the lipin substances were readily transferred to the flask by means of the water emulsion; the remainder was dissolved in chloroform, which was added in small amounts up to a total volume of 25 c c. With the volume of the emulsion so adjusted that it nearly ¾ filled the flask, 10 c c of concentrated hydrochloric acid were added from a pipet while the contents of the flask were being rotated and thoroughly mixed. This usually caused a complete breaking up of the emulsion, the lipins settling to the bottom of the flask in the chloroform solution. The flask was then filled to the mark and allowed to stand until the lipins had settled out leaving a clear fluid. The supernatant acid fluid was then filtered through paper, care being taken to prevent the chloroform solution from leaving the flask. The lipin-chloroform mixture was then dissolved in hot 95% alcohol and made up to volume. This alcoholic solution of the lipins is the one which was subsequently used for the determination of cholesterol, lecithins and the iodin number. The acid solution obtained by decantation from the lipin chloroform mixture constitutes what is here called the water-lipin fraction. The volume of this fraction was noted and phosphorus and nitrogen determinations made on aliquot parts of it. The values so obtained were used in making corrections for the water-lipin solution retained by the lipin-chloroform mixture. All the phosphorus present in this water-lipin solution was considered to be inorganic phosphorus. With some of the specimens of normal tissue, the entire lipin fraction was not dried to constant weight, but instead an aliquot part, usually $\frac{1}{10}$, was used for this purpose and a similar fraction was removed for determination of the iodin number. The remainder was evaporated to a syrup on the water bath and then emulsified with water as above indicated.

(*c*) The alcohol-ether-insoluble fraction consists of the dried residue left after extraction with alcohol and ether.

Small amounts of this residue were used for ash, calcium, total nitrogen and total phosphorus determinations. The remainder which constituted 0.7 or 0.8 of the entire amount was preserved for subsequent extraction with water.

(*d*) Water-soluble fraction.

The water-lipin solution was neutralized with sodium hydroxid and a definite portion of this used to extract a corresponding portion of the alcohol-ether residue. Either 0.7 or 0.8 of the total amount was used in each case. The neutralized water-lipin solution was divided into 2 equal parts and each in turn was used to extract the alcohol-ether residue. This extraction was accomplished in a shaking machine, each period of shaking lasting for 2 hours. Two subsequent extractions were, likewise, made using each time approxi-

mately 300 c c of distilled water. The suspended particles were removed from these extraction fluids by centrifugalizing. All four of these fluids were united, made slightly acid with acetic acid and evaporated to 1,000 c c. This constitutes the so-called water-soluble fraction, aliquot parts of which were used for the determination of the different forms of nitrogen and of phosphorus.

(c) Water-insoluble fraction.

This fraction is made up of the residue left after the completion of the alcohol, ether, and water extractions. It was used for the determination of total nitrogen, total phosphorus, phosphoprotein phosphorus, ash, calcium, and finally for the determination of the purin nitrogen.

ANALYTICAL METHODS

The determinations of cholesterol were made by Corper's method[26] while lecithin was estimated by the method of Koch and Woods,[27] the phosphorus being determined finally as $Mg_2P_2O_7$. The lecithin value is obtained by multiplying that of the phosphorus by the factor 25.75 on the assumption that the molecular weight of the lecithin is approximately 800. The iodin numbers of the lipin fractions were obtained by the use of von Hübl's iodin solution, the excess of iodin being titrated with 0.1 N $Na_2S_2O_3$. All total nitrogen determinations were made by the Kjeldahl method. For the estimation of the proteoses in the water soluble fraction, the proteoses were precipitated by saturation with $ZnSO_4$ in a solution made acid with H_2SO_4. The amount of nitrogen in this precipitate was then determined by the Kjeldahl method. The free amino-acids in the water-soluble fraction were estimated by the Van Slyke method.[28] For the peptones and peptids, a part of the solution was completely hydrolyzed and the total amino-acid content determined; from this total amino-acid nitrogen, the nitrogen of the free amino-acids and of the proteoses was subtracted. The negative value which was often obtained when these deductions were made serves to illustrate the unsatisfactory nature of this method of nitrogen partition. For all total phosphorus determinations, a Neumann combustion[29] was performed and the phosphorus ultimately determined gravimetrically as $Mg_2P_2O_7$.

The inorganic phosphorus was precipitated from the water soluble fraction by means of magnesia mixture, and the phosphorus determined gravimetrically as in other cases. An attempt was made to split off the phosphoprotein phosphorus in the water insoluble frac-

[26] Jour. Biol. Chem., 1912, 12, p. 197.
[27] Jour. Biol. Chem., 1905-6, 1, p. 203.
[28] Jour. Biol. Chem., 1913, 16, p. 121; and 1915, 23, p. 408.
[29] Ztschr. f. physiol. Chem., 1904, 43, p. 32.

tion by means of the action of 1% NaOH. From the solution so obtained, the phosphorus was determined in the same way as the inorganic phosphorus. The ash obtained from the alcohol-ether residues and from the water-insoluble fractions was analyzed for calcium by McCrudden's method.[30] The greater part of the water-insoluble fraction was completely hydrolyzed with 5% H_2SO_4, and the purin nitrogen estimated by the method of Krüger and Salomen.[31]

RESULTS OF ANALYSES

A comparison was made of the water content and dry weight of the normal tissues with the water content and dry weight of the caseous material and the walls of the tubercles arising from these tissues.

TABLE 1

BOVINE LYMPH GLANDS AND LYMPH GLAND TUBERCLES

Source of Tissue	Nature of Specimen	Water Content % of Moist Weight	Dry Weight % of Moist Weight
Peribronchial lymph glands	Normal	82.37	17.63
Mesenteric lymph glands	Normal	81.44	18.56
Mesenteric lymph glands	Normal	81.74	18.26
Peribronchial	Walls of tubercles	79.95	20.05
Peribronchial	Walls of tubercles	78.56	21.44
Peribronchial	Walls of tubercles	79.41	20.59
Mesenteric lymph glands	Walls of tubercles	79.51	20.49
Mesenteric lymph glands	Walls of tubercles	79.28	20.72
Peribronchial and mesenteric	Caseous material	75.16	24.84

From Table 1, it is seen that, in every case, the water content the normal lymph glands is distinctly higher than that of the tuberculous tissues. The lower water content of the walls of these lymph gland tubercles is, doubtlessly, due in part to the replacement of the succulent normal tissue by a dense fibrous connective tissue, and, in part also, to the early deposition of calcium salts in the caseous material included within these tissues. Even the semifluid caseous material from the lymph gland tubercles has a distinctly lower water content than either the normal lymph glands or the walls of the tubercles. This is readily understood, however, when it is found that this caseous material has an ash content of about 25% of the dry weight. The presence of the heavy inorganic salts accounts for the high value for the dry weight and the corresponding low water content.

[30] Jour. Biol. Chem., 1911, 10, p. 187.

[31] Hoppe-Seyler-Thierfelder, Handbuch d. physiol. u. pathol. chem. Analyse, 1909, p. 188.

As compared with normal bovine lymph gland tissue, the water content of normal bovine liver is distinctly lower, the average in the lymph glands analyzed being 81.85% as contrasted with an average of 70.68% for liver tissue. This value for the liver is distinctly lower than that obtained from the dense fibrous walls of lymph gland tubercles, so it might be expected that when liver tissue is destroyed in tubercle formation and new connective tissue takes its place, that the water content of the tubercle walls would be higher than that of the normal tissue. This is found to be the case. The average water content of the tubercle walls was 76.93% as compared with 70.68% for the normal liver tissue. In the walls of the lymph gland tubercles, the percentage of water present was 79.35, a value approximating that obtained for the walls of liver tubercles much more closely than do the percentages obtained for the two normal tissues.

TABLE 2

WATER CONTENT AND DRY WEIGHT OF NORMAL BOVINE LIVER COMPARED WITH THE WATER CONTENT AND DRY WEIGHT OF THE CASEOUS MATERIAL AND WALLS OF TUBERCLES FROM BOVINE LIVERS

Source of Tissue	Nature of Specimen	Water Content % of Moist Weight	Dry Weight % of Moist Weight
Liver	Normal	70.60	29.40
Liver	Normal	70.67	29.33
Liver tubercles	Walls of tubercles	77.07	22.93
Liver tubercles	Walls of tubercles	76.80	23.20
Liver tubercles	Caseous material	73.00	27.00
Liver tubercles	Caseous material	74.73	25.27
Liver tubercles	Caseous material from large tubercles	78.45	21.55

The amount of water in the caseous material from the liver tubercles varies considerably in the different specimens, the extreme variation being from 73.00-78.45%. This latter value was obtained with the caseous material from only the largest of the liver tubercles, all of which were over 2 cm. in diameter. The caseous centers of these large tubercles were observed to be much more fluid than the smaller ones. The average water content for the 3 specimens of caseous material from the liver tubercles is 75.39% which agrees very closely with the percentage of water present in the single specimen of caseous material from lymph gland tubercles, namely, 75.16.

The average total lipin content obtained for the 3 separate determinations on normal bovine lymph gland tissue is 24.39% of the dry weight, or 4.4% of the most weight. The peribronchial lymph glands

gave a somewhat lower value for the total lipins than that obtained from the mesenteric glands. This difference may be explained, at least in part, by the fact that the surrounding fat could be more completely removed from the peribronchial glands. The surfaces of the mesenteric glands were always distinctly oily even after a careful removal of the closely clinging fat tissue.

TABLE 3

TOTAL LIPIN FRACTION FROM NORMAL BOVINE LYMPH GLANDS AND FROM LYMPH GLAND TUBERCLES

Source of Tissue	Nature of Specimen	Moist Weight of Specimen	Dry Weight of Specimen	Dry Weight in % of Moist Weight	Total Lipins		
					Weight in Grams	% of Dry Weight	% of Ash-free Residue
Peribronchial lymph glands.....	Normal	74.4	13.117	17.63	2,980	22.72	23.81
Mesenteric lymph glands.....	Normal	100.7	18.730	18.60	4.588	24.50	25.80
Mesenteric lymph glands..... (Long form)	Normal	100.7	18.317	18.19	4.756	25.96	27.29
Peribronchial lymph glands.....	Walls of tubercles	102.8	20.61	20.05	6.140	29.79	32.61
Peribronchial lymph glands.....	Walls of tubercles	102.4	21.08	20.59	6.268	29.73	32.92
Peribronchial lymph glands.....	Walls of tubercles	106.9	22.92	21.44	6.607	28.80	31.26
Mesenteric lymph glands.....	Walls of tubercles	101.2	20.74	20.49	6.180	29.80	32.21
Mesenteric lymph glands.....	Walls of tubercles	63.3	13.12	20.72	3.830	29.19	31.44
Peribronchial and mesenteric lymph glands	Caseous material	87.8	21.81	24.84	4.250	19.49	27.05

The fat content of the tubercle walls is strikingly constant in the 5 specimens here studied, the maximum variation being 1% of the dry weight. The average fat content of the walls of these lymph gland tubercles is 29.46% of the dry weight, as compared with 24.39% for normal lymph glands. This is an increase of more than 20%. This finding, if considered alone, would seem to substantiate the claim made on the basis of fat staining that it is the cells at the boundary of the necrotic portion of the tubercle which are especially rich in fat. This relationship is not at all definite in the liver tubercles. The caseous material from the lymph gland tubercles, on the other hand, is found to contain a much lower percentage of lipins than occurs in the walls of these tubercles. The total lipins in this specimen constituted 19.49% of the dry weight, while the average for the tubercle walls was 29.46%, or approximately ½ more. The lipin content of the caseous material was even distinctly less than was that of the normal lymph gland tissue, the average for the normal tissue being 24.39% as compared with 19.49% in the caseous material.

This is a decrease of 20% below the normal value when the calculations are made on the dry weight. However, the dry weight of the caseous material is made up in large part of calcium salts. The ash content of this specimen is about 28% as compared with 5% in the normal tissue, while the nitrogen content of the alcohol-ether insoluble fraction of the caseous material is only 67% of the nitrogen content of the normal tissue. If the calculations were made on the basis of the organic constituents of the caseous substance, there would be an actual increase in lipins in the caseous material when compared with normal lymph glands.

TABLE 4

TOTAL LIPIN CONTENT OF NORMAL BOVINE LIVER AND LIVER TUBERCLES

Source of Tissue	Nature of Specimen	Moist Weight of Specimen	Dry Weight of Specimen	Dry Weight in % of Moist Weight	Total Lipins		
					Weight in Grams	% of Dry Weight	% of Ash-free Residue
Liver	Normal	101.0	29.694	29.40	10.204	34.36	35.07
Liver	Normal	100.1	29.249	29.22	9.762	33.38	34.16
Liver	Normal	101.2	29.682	29.33	10.329	34.77	35.55
Liver tubercles	Walls of tubercles	167.6	38.421	22.93	5.848	15.23	16.68
Liver tubercles	Walls of tubercles	105.4	24.453	23.20	3.939	16.11	17.68
Liver tubercles	Caseous material	109.1	29.457	27.00	5.147	17.47	24.70
Liver tubercles	Caseous material	109.3	27.620	25.27	4.875	17.65	23.70
Liver tubercles	Caseous material large tubercles	101.7	21.916	21.55	3.971	18.12	22.74

The total lipin content of normal bovine liver forms a higher percentage of the dry weight than in normal lymph glands. The average of 3 determinations on specimens all of which came from the same liver was 34.17% of the dry weight. Three parallel determinations made on specimens from another bovine liver gave a total lipin content of 31.28%. For the 2 livers the mean value is 32.72% of the dry weight, or 9.6% of the moist weight. When compared with the normal tissue, the walls of the liver tubercles have a remarkably low content of fatty material. The average for the 2 determinations made is 15.67% of the dry weight, or less than half the amount of fat obtained from the normal tissue. This result forms a striking contrast with that obtained by extraction of the walls of lymph gland tubercles, in which the fat content was definitely higher than in the normal lymph gland tissue. A more marked contrast appears when

one compares the lipin content of the walls of the tubercles arising from livers and from lymph glands. The value obtained for the fatty fraction derived from the walls of the lymph gland tubercles is 29.46% of the dry weight; that of the walls of the liver tubercles is only 15.67%, or not much more than half as much. It is worthy of note that the walls of the tubercles from these two sources have, in each case, approximately the same dry weight and ash content, so that the difference in total lipins cannot be explained by the more abundant deposition of inorganic salts in the walls of the liver tubercles. Of the 3 specimens of caseous material, the one coming from the largest liver tubercles contains the highest percentage of fatty material, namely, 18.12% of the dry weight. This slight difference is overbalanced, however, by the fact that the ash content of this caseous material is much lower, indicating a lesser content of inorganic salts.

TABLE 5

RESULTS OF THE ANALYSES OF THE TOTAL LIPIN FRACTIONS OF NORMAL BOVINE LYMPH GLANDS AND LYMPH GLAND TUBERCLES

Source of Tissue	Nature of Specimen	Total Lipins, % of Dry Weight	Cholesterol		Lecithin		Iodin No. after Acid Precipitation	Total N, % of Lipins
			% of Total Lipins	% of Dry Weight	% of Total Lipins	% of Dry Weight		
Peribronchial lymph glands	Normal	22.72	7.93	1.80	36.05	8.49	32.8	0.92
Mesenteric lymph glands	Normal	24.50	5.73	1.40	34.76	8.52	33.9	0.89
Mesenteric lymph glands (long form)	Normal	25.96	5.89	1.53	26.01	6.75	29.9	1.06
Peribronchial lymph glands	Walls of tubercles	29.79	13.52	4.03	25.78	7.68	44.0	1.05
Peribronchial lymph glands	Walls of tubercles	29.73	13.08	3.88	24.98	7.43	45.1	1.12
Peribronchial lymph glands	Walls of tubercles	28.80	13.16	3.84	24.21	7.07	44.0	1.06
Mesenteric lymph glands	Walls of tubercles	29.80	12.30	3.67	26.52	7.98	42.8	0.91
Mesenteric lymph glands	Walls of tubercles	29.19	13.78	3.96	27.55	8.04	41.8	1.09
Mesenteric and peribronchial lymph glands	Caseous material	19.49	26.58	5.18	12.10	2.36	51.2	0.53

As determined by Corper's method, the cholesterol present in the fatty fraction from normal lymph glands represents 6.52% of it, or 1.58% of the dry weight of the specimen. The single specimen of normal peribronchial lymph glands gave a higher cholesterol value than did the specimens of mesenteric glands. From all the specimens of walls of lymph gland tubercles, the amount of cholesterol obtained remained remarkably constant, varying from 12.30-13.78% of the

total lipin fraction. The average for the 5 specimens is 13.17% of the fatty substances, or 3.88% of the dry weight. When compared with the total lipin fraction of normal lymph glands, this percentage is almost exactly twice as much, and at the same time it forms twice as large a percentage of the dry weight, indicating an actual rather than simply a relative increase in cholesterol content. This high cholesterol value for the tubercle walls is explained, at least in part, by the appreciable amounts of caseous material which could not be removed from the fibrous walls and was, therefore, included with them. This caseous material itself contains a much larger percentage of cholesterol than do the walls of the tubercles. In the single specimen here analyzed, the cholesterol constituted 26.58% of the total fatty fraction, or 5.18% of the dry weight. This is over 3 times the amount of cholesterol in normal lymph glands when calculated on the basis of dry weight.

In the estimation of the lecithin in normal bovine lymph glands, the value obtained from the specimen consisting of the long form of mesenteric glands fails to agree with the results obtained with the other specimens, and there is every reason to believe that it is distinctly too low. However, using the average of the 3 determinations, the percentage of lecithin in the total lipin fraction is 32.27, which is equivalent to 7.92% of the dry weight. In the walls of these lymph gland tubercles, the average lecithin value is 25.81% of the fatty fraction, or 7.64% of the dry weight. This is a slight but not significant decrease below that of the normal tissue. The caseous material from these tubercles contained a much smaller percentage of lecithin. It constituted only 12.10% of the total fatty fraction, or 2.36% of the dry weight. Comparing this with the results obtained with the normal tissues, the fatty substances from the caseous material contain only ⅜ as much lecithin as do the fats from the normal tissues, while on the basis of dry weight they form even a smaller relative fraction. The slight decrease from the normal value noted in the walls of the tubercles may depend in part on the caseous material included in the tubercle walls.

Iodin number determinations were made on portions of the alcoholic solution of the lipins after precipitation of the fats from the water emulsion by means of acid chloroform. While the values given in the table may have some value for the sake of comparison, they do represent the true iodin numbers of the fats as they occurred in

the normal or in the tuberculous tissues. This is illustrated by the fact that the average of 3 iodin number determinations made on the fats from lymph glands, previous to the acid precipitation, was 41.1, while the same fats after precipitation and re-solution in alcohol gave an average iodin number of 32.2. Before this observation was made, however, all the iodin numbers had been determined on the specimens of tuberculous tissue subsequent to the precipitation of the fats in the acid solution. The iodin numbers obtained for the 5 samples of fats from tubercle walls are fairly constant and are uniformly distinctly higher than those obtained from the normal tissues. The average value is 43.5, as compared with 32.2 for the fats from the normal tissues when similarly treated. This difference is further accentuated when the fats of the caseous material are considered. The iodin number in this case was found to be 51.2.

TABLE 6

THE RESULTS OF THE ANALYSES OF THE TOTAL LIPIN FRACTIONS OF NORMAL BOVINE LIVER AND OF LIVER TUBERCLES

Source of Tissue	Nature of Specimen	Total Lipins, % of Dry Weight	Cholesterol		Lecithin		Iodin No. after Acid Precipitation	Total N, % of Lipins
			% of Total Lipins	% of Dry Weight	% of Total Lipins	% of Dry Weight		
Liver	Normal	34.36	2.81	0.97	39.91	13.71	43.4	0.74
Liver	Normal	33.38	4.11	1.37	43.00	14.35	38.9	1.07
Liver	Normal	34.77	4.31	1.50	40.69	14.15	40.2	0.90
Liver tubercles	Walls of tubercles	15.23	15.90	2.42	29.10	4.43	39.8	0.90
Liver tubercles	Walls of tubercles	16.11	12.97	2.09	28.33	4.56	40.3	1.16
Liver tubercles	Caseous material	17.47	27.20	4.75	16.74	2.91	42.1	1.02
Liver tubercles	Caseous material	17.65	26.05	4.61	15.71	2.78	46.0	0.93
Liver tubercles	Caseous material	18.12	26.20	4.75	15.45	2.80	42.3	0.88

Total nitrogen determinations were made on the lipin solutions for the purpose of showing how much nitrogen is carried over into th fraction other than that which can be accounted for by the amc of lecithin present. Calculated on the basis of 1 nitrogen atom i molecule having a molecular weight of approximately 800, the highe amount of lecithin found in any fatty fraction would account fc only 0.63% of nitrogen, while the amount actually determined was 0.92% of the total lipins. There is apparently an appreciable amount of nitrogen present in some undetermined form both in the lipi from normal and those from tuberculous tissues.

The cholesterol content of the total lipin fraction of normal bovine liver is here given as 3.74%, when the average of the three values is taken. For comparative purposes, this is probably too low since the first value given in the table is questionable because of failure to get satisfactory separation in the shaking out process.

An additional determination made on the fatty fraction from another normal liver gave the cholesterol content of the fats as 5.07%, or 1.60% of the dry weight. Including this value with those given above, the average becomes 4.07% of the total lipins, or 1.36% of the dry weight. Apparently cholesterol forms a somewhat smaller proportion of the liver lipins than it does of the lymph gland lipins, although on the basis of the dry weights there is no marked difference. The lipins from the walls of the liver tubercles are distinctly rich in cholesterol; it constitutes 14.4% of the fats, or 2.25% of the dry weight. A very much larger percentage of cholesterol, however, occurs in the lipins from the caseous material. For the 3 specimens, the average is 26.48% of the fatty fraction, or over ¼ of the entire amount. On the basis of the dry weight, the cholesterol is equal to 4.70%, or 3 times the amount obtained from normal liver. The variations in the lecithin are in the opposite direction as they were also in lymph gland tubercles. In the fats from the normal liver, lecithin constitutes 41.2%, or about 14% of the dry weight. The lipins from the walls of the tubercles contain 28.71% of lecithin, while those from the caseous material contain only 15.9%. Calculated for the dry weights, these values become 4.5% for the fats from the tubercle walls and 2.83% for those of the caseous material. Lecithin is, apparently, only about ⅜ as abundant in the lipins from the caseous material as in those from normal liver, and it constitutes only ⅕ as large a fraction of the dry weight.

The iodin number of the fats from normal liver is evidently somewhat higher than that of the fats from normal lymph glands, the rage obtained for the liver fats being 40.8 as compared with 32.2 the fats from lymph glands. For the 5 tuberculous specimens, the din number obtained is 42.1, or only a slight increase over that of he fats from normal liver.

The figures obtained for the nitrogen content of the lipins from ir liver tissues do not differ in any definite way from those previously not for the lymph gland lipins. There is no apparent tendency for

any larger amount of nitrogen to occur in the lipin fractions from the tuberculous tissues than from the normal tissues, other than that which can be accounted for by the other lecithin percentage.

TABLE 7

RESULTS OF THE ANALYSES OF THE ALCOHOL-ETHER INSOLUBLE FRACTIONS OF NORMAL LYMPH GLANDS AND OF LYMPH GLAND TUBERCLES

Source of Tissue	Nature of Specimen	Alcohol-Ether Residue								
		Alcohol-Ether Residue in % of Dry Wt.	Total N in %	Total N in % of Ash-free Residue	Total P in %	Ash.		Calcium		
						% of Alcohol-Ether Residue	% of Dry Wt.	% of Ash	% of Alcohol-Ether Residue	% of Dry Wt.
Peribronchial lymph glands	Normal	76.33	15.56	16,30	1.50	4.56	3.48	11.30	0.52	0.40
Mesenteric lymph glands	Normal	75.03	15.04	15.84	1.58	5.03	3.77	5.78	0.29	0.22
Mesenteric lymph glands (long form)	Normal	75.89	15.93	16.77	1.60	5.03	3.82			
Peribronchial lymph glands	Walls of tubercles	77.08	15.20	16.64	1.80	8.66	6.88	36.88	3.19	2.46
Peribronchial lymph glands	Walls of tubercles	76.18	13.84	15.32	2.38	9.68	7.38	47.35	4.58	3.49
Peribronchial lymph glands	Walls of tubercles	70.25	14.07	15.27	1.87	7.88	5.54	37.80	2.98	2.09
Mesenteric lymph glands	Walls of tubercles	74.44	14.52	15.70	1.83	7.48	5.57	35.20	2.63	1.96
Peribronchial & mesenteric lymph glands	Caseous material	80.80	10.41	14.45	9.88	27.95	22.58	56.27	15.73	12.71

The insoluble residue left after complete extraction of normal lymph glands with alcohol and ether averages in these specimens 75.75% of the dry weight of the tissue. The nitrogen content of this residue is 15.5%; the phosphorus present makes up 1.56% of its weight and the ash constitutes 4.87%. The amount of calcium in the ash is not great enough for accurate determination by the method used, but it constitutes, perhaps, 0.2-0.5% of the alcohol-ether residue. The residues from the walls of the lymph gland tubercles form about the same percentage of the total dry weight as in the normal tissues, the average is 74.5% as compared with 75.75% for normal glands. The percentage of nitrogen in the residues from the tubercle walls is 14.4, while in the normal tissue it is 15.5. This decrease in the percentage of nitrogen is relatively slight when compared with the increase in ash and in calcium. The ash increases from 4.87% in normal tissue to 8.4% in the tubercle walls, while the calcium increases from less than 0.5% to an average of 3.34% of the alcohol-ether residue. The change in the phosphorus content by no means parallels that of the ash.

Its increase is from 1.56-1.97% of the residue. If the amount of calcium found here is combined in the usual way with phosphoric and carbonic acids in the approximate ratio of 4:1, about 1.5% of the phosphorus present would be required to unite with the calcium, leaving only about 0.5% for the organic compounds.

The specimen of caseous material is conspicuous for its high ash and calcium content, in spite of the fact that there were no definitely calcified areas in any of these tubercles. The ash constituted 27.95% of the alcohol-ether residue, or calculated on the basis of the dry weight this is equivalent to 22.58%. The calcium itself made up 15.73%, or approximately ⅙ of the alcohol-ether residue, while the phosphorus constituted 9.88% of it.

TABLE 8

THE RESULTS OF THE ANALYSES OF THE ALCOHOL-ETHER INSOLUBLE RESIDUES FROM NORMAL LIVER AND FROM LIVER TUBRCLES

Source of Tissue	Nature of Specimen	Alcohol-Ether Residue								
		Alcohol-Ether Residue in % of Dry Wt.	Total N in %	Total N in % of Ash-free Residue	Total P in %	Ash		Calcium		
						% of Alcohol-Ether Residue	% of Dry Wt.	% of Ash	% of Alcohol-Ether Residue	% of Dry Wt.
Liver.....	Normal	68.19	14.95	15.26	0.64	2.03	1.39	19.05	0.39	0.27
Liver.....	Normal	69.25	14.91	15.26	0.69	2.28	1.58	15.80	0.36	0.25
Liver.....	Normal	67.07	15.27	15.51	0.69	2.19	1.47	22.20	0.44	0.30
Liver tubercles	Walls of tubercles	86.94	14.53	15.91	1.82	8.69	7.56	39.45	3.43	2.98
Liver tubercles	Walls of tubercles	88.18	14.61	16.04	1.86	8.90	7.85	43.79	3.90	3.44
Liver tubercles	Caseous material	83.76	10.15	14.35	5.06	29.27	24.52	54.29	15.89	13.31
Liver tubercles	Caseous material	85.49	10.66	14.31	4.82	25.53	21.65	51.07	12.93	11.05
Liver tubercles	Caseous material (large tubercles	82.82	13.32	16.71	3.59	20.30	16.81	50.50	10.25	8.49

In the 3 specimens of normal liver, the residues left after extraction with alcohol and ether average 68.16% of the dry weight.

Because of the low fat content in the walls of the liver tubercles, an especially high value is obtained for the alcohol-ether insoluble fraction. This forms 87.56% of the dry weight, as compared with 68.16% in the normal tissue. The residues from the caseous material form a slightly smaller percentage of the dry weight, than do the residues from the tubercle walls.

In the normal liver tissue, nitrogen constitutes about 15% of the alcohol-ether residue. Its amount is slightly lower in the walls of the

tubercles where it averages about 14.5%. In the 2 specimens of caseous material from medium-sized liver tubercles, nitrogen forms 10.4% of the residue, although the inorganic materials forming the ash make up 27.4% of this fraction. This value for the nitrogen if calculated on the basis of the organic substances present would form 14.3% of such compounds, which shows that there is no marked reduction in the amount of nitrogen in this caseous material below that which would be present in a corresponding amount of protein under normal conditions. The reduction in the amount of nitrogen is still less in the specimen of caseous material from the large liver tubercles. Here, it constitutes 13.2% of the alcohol-ether residue, although the ash in this case formed 1/5 of the entire weight of this fraction.

TABLE 9

THE RESULTS OF THE ANALYSES OF THE ALCOHOL-ETHER-WATER INSOLUBLE RESIDUES OF NORMAL LYMPH GLANDS AND LYMPH GLAND TUBERCLES

Source of Tissue	Nature of Specimen	Water Insoluble Residue										
		Water Insol. % of Dry Wt.	Total N in %	Total N in % of Ash-free Residue	Total P		Phospho-protein P, % of P	Ash		Calcium		Purin N % of N
					% of Water Insol.	% of Dry Wt.		% of Water Insol.	% of Dry Wt.	% of Ash	% of Water Insol.	
Peribronchial lymph glands	Normal	67.97	16.12	16.36	0.60	0.41	0.14	1.44	0.98	13.04	0.19	0.32
Mesenteric lymph glands	Normal	69.43	15.52	15.85	0.95	0.66	0.18	2.11	1.46	11.69	0.25	0.57
Mesenteric lymph glands (long form)	Normal	68.79	16.01	16.30	1.02	0.70	0.16	1.78	1.22	6.67	0.12	0.44
Peribronchial lymph glands	Walls of tubercles	72.68	14.46	15.47	1.52	1.10	0.53	6.50	4.72	58.79	3.82	0.21
Peribronchial lymph glands	Walls of tubercles	68.07	14.44	15.80	1.37	0.93	0.65	8.61	5.86	46.08	3.97	0.26
Peribronchial lymph glands	Walls of tubercles	62.61	14.08	15.03	1.16	0.73	0.69	6.30	3.94	43.97	2.77	0.22
Mesenteric lymph glands	Walls of tubercles	63.97	15.65	16.43	1.34	0.86	0.88	4.73	3.03	28.57	1.35	0.28
Peribronchial & mesenteric lymph glands	Caseous material	78.22	10.64	15.81	5.07	3.97	2.04	32.68	25.56	41.13	13.44	0.06

The value obtained for the total phosphorus in the alcohol-ether insoluble fraction of normal liver is 0.67%. In normal lymph glands, the corresponding percentage was 1.56%. In the walls of the liver tubercles, the amount of phosphorus increased to nearly 3 times the amount found in the normal tissue, while the calcium present increased to nearly 10 times that obtained from normal tissue. An exceptionally high phosphorus content occurs in the residues from the caseous material. In these the phosphorus averaged 4.49%, or 7 times that

of normal liver. With this increase in phosphorus, there is much more than a corresponding increase in the amount of ash. For the normal liver, the ash value is about 1.5%, while in the caseous residues, it averages approximately 21%, or 14 times the amount in the normal tissue. The increase in calcium more than parallels the increase in ash; from a normal of 0.4%, it increases to 3.6% in the tubercle walls, and reaches 13% in the caseous residues.

A comparison of the alcohol-ether residues of the specimens of normal lymph glands with the water insoluble fractions makes evident the fact that only a small percentage of the residues goes into solution in water at room temperature. In the water insoluble fraction, there is a decrease of 7.02% of the dry weight below the percentage of the alcohol-ether residue. In the specimens from the walls of the lymph gland tubercles, the decrease is 7.65%, and for the caseous material 2.58%. The water soluble materials form, apparently, a smaller percentage of the caseous substances than they do of the normal tissues or of the tubercle walls. In nearly every case, the total nitrogen present in the water insoluble residues constitutes a slightly higher percentage than it does of the alcohol-ether residues. The loss in weight is evidently due to the solution of substances relatively poorer in nitrogen than those which remain. As a result of the extraction with water, the residues suffer a loss in their phosphorus content. This is a decrease of approximately 45% in the normal tissues and about 40% in the tuberculous specimens. That portion of the phosphorus which is split off from the residues by 1% NaOH is here listed as phosphoprotein phosphorus. In the normal tissues this constitutes about $\frac{1}{5}$ of the total phosphorus, while in the tuberculous specimens it forms a much larger part of the total phosphorus, sometimes even more than half. These high values are, doubtlessly, due to a solution of a part of the inorganic phosphorus when the alkaline solution is neutralized with acetic acid. The percentages of ash and calcium are usually somewhat lower in the water insoluble fractions than in the alcohol-ether residues. The determinations of purin nitrogen in the water insoluble residues of normal lymph glands gave an average of 0.44% of these residues, while there was a distinctly smaller amount in the tubercle walls where the percentage was 0.24%. The single specimen of caseous material from lymph glands seemed to contain only a trace of purin nitrogen, the value obtained being 0.06% of the water insoluble fraction. This low content of purin nitrogen in

caseous material is not surprising, but it stands out in striking contrast to the exceptionally high percentages obtained from the caseous material from liver tubercles.

TABLE 10

THE RESULTS OF THE ANALYSES OF THE WATER-INSOLUBLE RESIDUES FROM SPECIMENS OF NORMAL LIVER AND FROM LIVER TUBERCLES

Source of Tissue	Nature of Specimen	Water Insoluble Residue										
		Water Insol. % of Dry Wt.	Total N in %	Total N in % of Ash-free Residue	Total P		Phospho-protein P, % of P	Ash		Calcium		Purin N % of N
					% of Water Insol.	% of Dry Wt.		% of Water Insol.	% of Dry Wt.	% of Ash	% of Water Insol.	
Liver.....	Normal	65.91	15.20	15.26	0.25	0.17	0.09	0.39	0.26			0.15
Liver.....	Normal	65.48	15.12	15.17	0.27	0.18	0.06	0.34	0.22			0.14
Liver.....	Normal	64.17	15.22	15.39	0.24	0.16	0.06	1.08	0.71	55.56	0.60	0.10 0.11
Liver tubercles	Walls of tubercles	81.52	13.99	15.56	2.26	1.84	1.03	10.08	8.22	54.39	5.49	0.16
Liver tubercles	Walls of tubercles	81.18	14.17	15.43	1.51	1.23	0.83	8.19	6.65	49.49	4.05	0.16 0.14
Liver tubercles	Caseous material	76.63	10.64	15.14	5.34	4.09	2.34	29.75	22.80	59.08	17.58	0.26
Liver tubercles	Caseous material	80.14	10.97	15.07	4.57	3.66	2.39	27.21	21.81	61.71	16.79	0.27
Liver tubercles	Caseous material (large tubercles)	79.66	13.05	15.48	3.58	2.85	2.15	15.69	12.50	40.15	6.30	0.27

In the specimens of normal bovine liver, the water insoluble fractions form 65.18% of the dry weight, while the alcohol-ether residues constitute 68.17%. As a result of the extraction of these residues with water, the amount which goes into solution, together with a small mechanical loss, is 3% of the dry weight of the specimen.

From the residues of the tubercle walls, 6.21% of the dry weight passed over into the water soluble fraction, and from the residues of the caseous material, 5.21%. Here, as in the lymph gland tissues, the percentage of nitrogen in the water insoluble residues is slightly higher than in the alcohol-ether residues. In the normal specimens the total phosphorus is reduced to less than half that of the alcohol-ether residues, but there is no corresponding reduction in the phosphorus in the specimens from tuberculous tissues; in these, the phosphorus values remain practically unchanged. The amount of ash, likewise, is decreased in the normal specimens, but remains nearly constant in the tuberculous residues. Calcium, as a rule, forms a larger percentage of the water insoluble fraction than it does of the alcohol-ether residues. The average value obtained for the purin

nitrogen in the residues of normal liver is 0.13% of the water insoluble fraction; in the residues from the tubercle walls, a similar percentage is obtained, namely, 0.15. Very strangely, three closely agreeing determinations of purin nitrogen made on the residues of caseous material from liver tubercles gave a distinctly higher purin content than that of normal liver or of the liver tubercle walls. Here, the percentage obtained was 0.27, as compared with 0.13 for normal liver and 0.15 for the tubercle walls.

TABLE 11

THE RESULTS OF THE ANALYSES OF THE WATER SOLUBLE FRACTION OF NORMAL BOVINE GLANDS AND LYMPH GLAND TUBERCLES

Source of Tissue	Nature of Specimen	Water Soluble Fraction						
		Nitrogen					Phosphorus	
		Total N in % of Dry Weight	Proteose N in % of Total N	Ammonia N in % of Total N	Free Amino-Acid N in % of Total N	Amino-Acid N in % of Total N	Total P in % of Dry Weight	Inorganic P in % of Total P
Peribronchial lymph glands	Normal	1.59	7.81	7.38	21.10	27.59	0.88	54.54
Mesenteric lymph glands	Normal	1.28	9.03	1.00	23.59	36.48	0.74	56.20
Mesenteric lymph glands	Normal	1.41	9.37	Trace	14.33	30.85	0.66	50.21
Peribronchial lymph glands	Walls of tubercles	0.89	16.90	7.48	21.40	28.33	0.50	78.83
Peribronchial lymph glands	Walls of tubercles	0.88	12.90	5.16	15.80	33.12	0.53	88.26
Peribronchial lymph glands	Walls of tubercles	0.83	11.65	2.22	28.68	38.20	0.48	85.89
Mesenteric lymph glands	Walls of tubercles	1.66	24.58	7.80	18.61	36.40	0.59	80.21
Peribronchial and mesenteric lymph glands	Caseous material	0.26	35.20	Lost	14.89	26.80	0.20	93.50

The total nitrogen in the water soluble fraction from the specimens of normal lymph glands is equivalent to 1.43% of the dry weight of the specimens. The percentage of nitrogen in the corresponding fractions from the walls of the peribronchial lymph gland tubercles is, in each case, definitely lower than in the normal tissues, the average being 0.87% instead of 1.43%. The tubercle walls from the mesenteric lymph gland tubercles appear, from Table 11, to be exceptional in their high content of water soluble nitrogen, but this is explained by the fact that a clear solution was not obtained by centrifuging and some protein material was carried over in suspension.

The amount of water soluble nitrogen in the specimen of caseous material is exceptionally low, constituting only 0.26% of the dry

weight, or less than ⅕ of that derived from the normal tissue. There seems to be a rather definite increase in the proteose nitrogen in the tuberculous as compared with the normal tissues, the caseous material being the richest in this form of nitrogen compounds. The values obtained for ammonia nitrogen are quite inconstant, as are those, also, for the free amino-acids. The attempt to determine a peptone nitrogen fraction, following acid hydrolysis of samples of the water soluble substances, was eminently unsatisfactory. The value obtained for the peptone nitrogen was often a negative one. A slightly smaller percentage of phosphorus, when calculated on the basis of the dry weight, goes into the water solution from the tuberculous than from the normal tissues. This difference is most marked in the specimen which was completely caseous. On the other hand, of the phosphorus which does enter the water fraction, an increasing large percentage of it is inorganic phosphorus in the specimens of tuberculous tissues.

TABLE 12

THE RESULTS OF THE ANALYSES OF THE WATER SOLUBLE FRACTIONS OF NORMAL BOVINE LIVER AND OF LIVER TUBERCLES

Source of Tissue	Nature of Specimen	Water Soluble Fraction						
		Nitrogen					Phosphorus	
		Total N in % of Dry Weight	Proteose N in % of Total N	Ammonia N in % of Total N	Free Amino-Acid N in % of Total N	Amino-Acid N in % of Total N	Total P in % of Dry Weight	Inorganic P in % of Total P
Liver	Normal	0.75	5.44	2.43	18.35	32.20	0.49	66.25
Liver	Normal	0.81	2.11	7.11	11.50	23.84	0.56	58.91
Liver	Normal	0.96	4.27	0.42	9.21	29.49	0.59	57.61
Liver tubercles	Walls of tubercles	0.68	39.19	5.10	16.10	41.45	0.26	77.58
Liver tubercles	Walls of tubercles	0.71	37.45	5.42	18.23	34.80	Lost	Lost
Liver tubercles	Caseous material	0.24	13.37	10.72	37.46	40.05	0.17	84.51
Liver tubercles	Caseous material	0.23	26.12	9.87	38.38	43.30	0.14	Lost
Liver tubercles	Caseous material (Large tubercles)	0.29	14.01	Lost	23.57	27.87	0.21	80.88

In liver, as well as in lymph gland specimens, the higher percentage of water soluble nitrogen is obtained with normal rather than with tuberculous specimens. The difference, in this respect, between normal liver and the walls of liver tubercles is slight, but only about ⅓ as much nitrogen goes into solution from the caseous material. The ammonia nitrogen fraction is apparently somewhat increased in the

caseous material from liver tubercles, as is also the free amino-acid content. The value obtained for peptone nitrogen is negative in every specimen of tuberculous liver, although it has a positive value in the normal tissues. The phosphorus entering the water solution from the tuberculous tissues, in every case, constitutes a smaller percentage of the dry weight than with normal tissues.

Here, again, an increasingly large percentage of the total phosphorus is inorganic phosphorus in the water solutions from the tuberculous tissues.

THE ANALYSIS OF CASEOUS MATERIAL FROM HUMAN LYMPH GLAND TUBERCLES

This specimen consisted of the caseous material from 3 tracheobronchial lymph glands. The largest of these glands was about 3.5 cm. in its greatest dimension and it was completely caseous without any definite areas of calcification in it, other than fine sandlike particles. The two smaller glands were caseous and partly calcified. The entire specimen weighed only 13.5 gm. and was, therefore, too small for accurate analysis.

In spite of the fact that most of the specimen formed a semifluid mass, its dry weight was 60.7% of the moist weight, or the water present formed only 39.3% of the original weight. From the entire specimen 0.45 gm. of lipins was obtained, which is equivalent to 5.5% of the dry weight, or 19.7% of the ash-free residue. Unfortunately, the cholesterol was lost. Lecithin was found to constitute 30.9% of the total lipins, a percentage 2½ times as great as that of the fats from bovine caseous material. This lecithin value represents 1.7% of the dry weight, or 6.1% when calculated on the ash-free basis.

The iodin number of these fats was found to be 30.7, which is about the same as that obtained for the fats from normal bovine glands, but much lower than that from the single specimen of caseous material.

The alcohol-ether residue of this specimen formed 93% of the dry weight, and the water-insoluble fraction 88.9%. The total nitrogen determinations made on the alcohol-ether residue and on the water-insoluble fraction gave 2.46 and 2.33% of nitrogen, respectively. When the ash content of these residues has been deducted, these nitrogen values become 10.93 and 10.84%. This represents a reduc-

tion of the organic substances far below that seen in any of the specimens of caseous material from bovine tissues. The total phosphorus content of each of these residues was 9.25-9.50%, while the ash constituted 77.5-78.5%. Of this ash the calcium formed over 60% of its weight. An attempt was made to evaluate the purin nitrogen, but evidence of only a trace of purins was obtained.

DISCUSSION OF RESULTS

The results of the analyses of normal bovine lymph glands agree closely, so far as they are comparable, with those obtained by Bang.[7] For the water content and dry weight of mesenteric glands of oxen, he reported 80.41% of water and 19.59% of solids, as compared with the figures here given of 81.59% of water and 18.41% of solids for glands having the same origin. There is a similar close agreement on the percentage of fatty substances present. Bang gave the alcohol soluble substances as 4.76% of the fresh weight; they are here reported as constituting 4.49%, or the equivalent of 24.39% of the dry weight. The percentage of ash given by Bang is 1.05%, a value somewhat higher than that obtained in these analyses. The water content of normal bovine liver as given by v. Bibra[1] is 71.39% of the moist weight and the average of Oidtmann's determinations is 71.66%. For the two livers examined in these analyses, the water content was found to be 70.63%, leaving a dry weight of 29.37%. No such close agreement exists with regard to the fat content of bovine livers. v. Bibra gives percentages of 2.64 and 3.28, based on the fresh weight, or when calculated on the dry weight, the average is 10.35. Profitlich found the fat to vary from 10.87-21.78% of the dry weight. In the two normal livers which I have examined, the total lipins constituted 31.28 and 34.17% of the dry weight, or an average of 32.72%. There was no macroscopic evidence of pathologic fatty changes in either of these livers and the higher percentages obtained are probably due to the method of extraction.

In the tuberculous tissues, the finding of a higher fat content in the walls of lymph gland tubercles than in the completely caseous material agrees with the observations made by Wells in regard to the scrapings from tubercle walls, but differs in the fact, that in this caseous material both calcium and phosphorus were present in much larger amounts than in the tubercle walls, so that a part of the decrease in total lipins may be attributed to the deposition of calcium salts.

His finding that the water soluble fraction of the caseous liquid content of tubercles constitutes a smaller percentage of the dry weight than it does in the scrapings from the walls is confirmed by these analyses. A similar low lipin content was found for the caseous material from human lymph glands, but here there is a correspondingly great increase in inorganic salts and a like decrease in the protein constituents. Bossart obtained about the same amount of fat from pure caseous material from human lymph glands as was obtained here for the caseous material of bovine origin. The values which he reports for his partially caseous specimens are lower, however, than those reported here for the walls of tubercles or for normal lymph gland tissue. According to Bossart's analyses, cholesterol apparently made up a larger percentage of the total fats in the partially caseous material than it did in the completely caseous specimen. In bovine lymph glands and livers, cholesterol seems to constitute a much larger percentage of the fats from the caseous material than it does of the fats from the walls of tubercles or from normal tissues.

The variation in the amount of lecithin is in the opposite direction. It is more abundant in the specimens of normal tissue than it is in the tuberculous ones and the amount in the caseous material constitutes the smallest percentage of the total fats, as well as of the dry weight. This finding of a decrease in the lecithin content of the fats from the caseous material harmonizes with the similar finding by Wells in his study of the fats of livers in acute yellow atrophy and in delayed chloroform poisoning, and also with the observation made by Griniew on the organs of tuberculous guinea-pigs.

So far as lymph gland tubercles are concerned, the results obtained by these analyses seem to support the evidence furnished by staining methods that the walls of tubercles contain a larger amount of fat than does the caseous material itself. While this does not hold true of liver tubercles, it seems quite likely that the difference is due to the more rapid formation of the tubercles in the liver tissue which is already extremely rich in fats. In all 4 specimens of caseous material, the total lipins constitute a smaller percentage of the dry weight than in the normal tissues from which this caseous material originated. This shows conclusively that caseous material is not so rich in fats as it has usually been considered.

The cholesterol of the total lipins increases at about the same rate that the lecithin decreases in the tuberculous tissues, so that the sum

of the 2 percentages remains practically constant, leaving the simple fats to form about the same percentage of the total lipins in normal and tuberculous tissues.

In the alcohol-ether-insoluble residues, the percentage of total nitrogen is slightly higher in the normal lymph gland residues than in those from normal liver, in spite of the fact that ash content of the lymph gland residues averages twice that of the normal liver residues. The walls of the tubercles arising from these 2 tissues give residues which agree more closely in their nitrogen content than do the tissues from which they arise, just as they also resemble each other more closely in their histologic structure. The residues from 3 of the specimens of caseous material give approximately the same percentage of nitrogen, and about the same percentage of ash, whether from lymph gland or from liver tubercles. In the residues from the caseous material of the large liver tubercles, the higher content of nitrogen is dependent in part on the smaller amount of inorganic salts present, and probably in part also on the more rapid necrosis than that which occurs in the formation of the smaller tubercles, so that less extensive changes have taken place in the proteins originally present in the area.

The total phosphorus content of the normal lymph gland residues averages twice that of the residues of the normal liver. This can be explained by the greater amount of nucleoproteins in the lymph glands. The walls of the tubercles arising in lymph glands or in bovine liver give residues which contain approximately the same amount of phosphorus. As compared with the phosphorus content of the normal tissues, the increase in the amount of phosphorus in the walls of the lymph gland tubercles is small as compared with the increase in the ash content. This is apparently due to a decrease in the nucleoproteins and their replacement by proteins poorer in phosphorus, together with the deposition of inorganic salts. In the residues from liver tubercles, the amount of phosphorus is increased to nearly 3 times the amount in normal tissue, although the total ash content is only slightly higher than that of the walls of lymph gland tubercles. In this case there was no tissue rich in nucleins to be replaced, so that the increase in the phosphorus is due chiefly to the deposition of inorganic salts.

In the water-insoluble residues of lymph glands and lymph gland tubercles, the purin nitrogen decreases with the tubercle formation

and reaches a minimum in the residues of caseous material. As lymph gland tissue is replaced by fibrous tissue relatively poor in nuclein substances, a decrease in purin nitrogen would be expected in the tubercle walls. Likewise, in caseation, as the nuclear substances disappear, as shown by staining methods, a further reduction of purin content probably also occurs. From the results obtained with residues from normal liver and from liver tubercles, the tubercle walls are apparently slightly richer in nucleoproteins than is the normal liver. A finding which, at present, cannot be explained is the distinctly greater purin content of the caseous residues of liver tubercles as compared with the purins in the residues of normal liver and in walls of liver tubercles. Three closely agreeing determinations give an average value ½ more than that of the tubercle walls and approximately twice that of normal liver tissue. This does not conform with the finding of an extremely low percentage of purin nitrogen in the single specimen of caseous material from bovine lymph gland tubercles.

SUMMARY

The water content of normal bovine lymph glands constitutes about 81 or 82% of the moist weight. No very distinct differences are noted between peribronchial glands and those from the mesenteric region. The tubercle walls and the caseous material from lymph gland tubercles contain a lower percentage of water than does the normal tissue.

In normal bovine liver tissue, the percentage of water present is less than that of the tubercle walls or of the caseous material from liver tubercles. The specimens of caseous material from lymph gland and liver tubercles approach each other closely in their water content, the average being about 75% for the bovine material.

The alcohol-ether-soluble substances from normal bovine lymph glands form about 24.4% of the dry weight, or about 4.4% of the moist weight. The walls of the lymph gland tubercles contain a distinctly larger amount of lipins than does the caseous material or the normal tissue. On the contrary, the walls of liver tubercles are poor in lipins as compared with the normal tissue, and they contain a smaller amount of fats than does the caseous material from these tubercles. When calculated on the basis of the dry weight, the caseous material from lymph gland tubercles contains a smaller percentage of lipins than does normal lymph gland tissue. When the ash is deducted, this difference disappears and the content of lipins becomes

equal to or slightly greater than that of the normal tissue, but less than that of the tubercle walls. When calculated on an ash-free basis, the lipin content of the caseous material from liver tubercles is distinctly less than that of the normal tissue but greater than the lipin content of the tubercle walls.

Cholesterol forms about 6.5% of the lipins from normal bovine lymph glands, or about 1.5% of the dry weight. The lipins from the walls of lymph gland and liver tubercles contain, in every case, 2-3 times as much cholesterol as do the lipins from the normal tissues. This is an actual increase also when calculated on the basis of the dry weight. The caseous material contains even a larger percentage of cholesterol than do the tubercle walls.

Lecithin constitutes about 32% of the lipin fraction of normal bovine lymph glands, or about 7.9% of the dry weight; the corresponding values for normal liver are 41.2% of the fats, or 14% of the dry weight. The lecithin content of the fats from the tubercle walls is slightly less than that of the normal tissues, while there is a very marked reduction in the lecithin content of the lipins from caseous material of bovine origin. In the specimen of caseous material from human lymph glands, lecithin formed 30.9% of the total lipins.

The iodin numbers obtained for the fats of the tuberculous specimens from lymph glands are higher than those from the normal tissues. This observation does not hold true for the liver specimens. In the latter, there is no difference noted between the iodin numbers obtained for the lipins from normal and tuberculous specimens, although the values are practically the same as those from the fats from the lymph gland tubercles.

In the residues of caseous material left after extraction with alcohol and ether, the nitrogen content remains relatively high, in fact, the reduction in nitrogen content is only slight when the calculations are made on ash-free residues. The percentage of nitrogen does not differ much from that obtained from the normal proteins of these tissues.

In specimens of caseous material in which there are no macroscopic evidences of calcification other than the presence of sandlike particles, calcium sometimes forms as much as 15% of the residue left after extraction of the fats. In such residues, the phosphorus content may reach 9%.

The amount of purin nitrogen in the walls of lymph gland tubercles is only slightly more than half that of normal lymph gland tissue, and the amount is apparently much less in the caseous material. In the residues from the walls of liver tubercles, purin nitrogen is present in only slightly higher percentage than in the normal liver. The results here obtained would seem to indicate that the purins are even more abundant in the caseous residues of liver tubercles.

The amount of material which enters the water solution during extraction is distinctly less from caseous material than from the residues of normal tissues.